

Elevated Atmospheric Carbon Dioxide and Weed Populations in Glyphosate Treated Soybean

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ABSTRACT

Although rising atmospheric carbon dioxide (CO_2) is known to stimulate the growth of agronomic weeds, the impact of increasing CO_2 on herbicide efficacy has not been elucidated for field-grown crops. Genetically modified soybean [*Glycine max* (L.) Merr.] (i.e., Round-up Ready soybean) was grown over a 2-yr period at ambient and projected levels of atmospheric carbon dioxide (CO_2 , 250 $\mu\text{mol mol}^{-1}$ above ambient), with and without application of the herbicide, glyphosate [$^{\wedge}$ -(phosphonomethyl)glycine], to assess the impact of rising atmospheric carbon dioxide concentration [CO_2] on chemical efficacy of weed control. For both years, soybean showed a significant vegetative response to elevated [CO_2], but no consistent effect on seed yield. For 2003, weed populations for all treatments consisted entirely of C_4 grasses, with no effects on weed biomass (unsprayed plots) or glyphosate efficacy (sprayed plots). However, in 2004, weed populations were mixed and included C_3 and C_4 broadleaves as well as C_4 grasses. In this same year, a significant increase in both C_3 broadleaf populations and total weed biomass was observed as a function of [CO_2] (unsprayed plots). In addition, a [CO_2] by glyphosate interaction was observed with significant C_3 broadleaf weed biomass remaining after glyphosate application. Overall, these data emphasize the potential consequences for CO_2 -induced changes in weed populations, biomass, and subsequent glyphosate efficacy in Round-up Ready soybean.

ATMOSPHERIC CARBON DIOXIDE concentration has shown an increase of about 21 % from 315 to 379 $\mu\text{mol mol}^{-1}$ since the late 1950s (cdiac.ornl.gov/trends/co2/sio-mlo.htm; verified 10 February 2006). Although the rate of increase is variable, levels are projected to exceed 600 $\mu\text{mol mol}^{-1}$ by the end of the 21st century (Houghton et al, 2001).

Overall, current and projected increases in global atmospheric [CO_2] are likely to change the biology of agricultural weeds in two fundamental ways. The first is related to climate stability. Evaluations by the Intergovernmental Panel on Climate Change (IPCC) based, in part, on an assessment by the U.S. National Academy of Sciences has indicated that the rise of [CO_2] and associated "greenhouse" gases could lead to a 1.4 to 5.8°C increase in global surface temperatures, with subsequent consequences on precipitation frequency and amounts (IPCC, 2001). Temperature and water availability remain key factors in determining weed species growth and success (Patterson and Flint, 1990; Patterson, 1995a). The second likely impact is the [CO_2] "fertilization" effect. That is, plants evolved at a time when the at-

mospheric [CO_2] appears to have been four or five times present values (Bowes, 1996). Because CO_2 remains the sole source of carbon for plant photosynthesis, and because at present, [CO_2] is less than optimal, as atmospheric [CO_2] increases, photosynthesis and growth will be stimulated accordingly. Although, in general, the relative effect of increasing [CO_2] is greater for C_3 than C_4 species, species-specific responses demonstrate a wide range of relative enhancement within C_3 and C_4 weeds (Patterson and Flint, 1980).

Weed management efforts, in turn, will be altered both by climatic uncertainty and rising carbon dioxide levels (Ziska, 2004). To date, our understanding of how rising CO_2 affects chemical weed management has focused exclusively on reductions in efficacy for individual weeds or monocultures (Ziska et al., 1999, 2004). Data on how elevated CO_2 could alter weed populations (and subsequent chemical control) are not available in genetically modified crops, that is, crops designed to be treated with herbicides. Our specific objective in the current study, therefore, was to quantify changes in weed populations and potential changes in chemical control efficacy as a function of [CO_2] for Round-up Ready (Monsanto Corp., St. Louis, MO) soybean grown with and without application of commercially formulated glyphosate.

MATERIALS AND METHODS

Experimental Treatments

The experiment was conducted over a 2-yr period at a 0.3-ha plot at Beltsville, MD. Field soil was classified as a Corduroy silt-loam (*Cordurus harbore*), pH 5.5 with high availability of potash, phosphate, and nitrate. Twelve experimental aluminum chambers (3 m in diameter and 2.25 m in height) covering an area of 7.2 m^2 were placed at regular intervals within the field. Because of the chamber size, a modified suspended chamber top was necessary to prevent wind intrusion and to maintain a stable CO_2 concentration. For each year of the study, individual chambers were assigned one of two CO_2 treatments, either ambient or ambient +250 $\mu\text{mol mol}^{-1}$ CO_2 ; and one of two herbicide treatments, either sprayed at manufacturers recommended dosage, or unsprayed. CO_2 treatments were maintained 24 h d^{-1} from germination until maturity. Air was supplied through perforations in the inner wall of the lower half of the chamber. Air was adjusted to the desired [CO_2] with pure CO_2 supplied from a 5 Mg liquid CO_2 tank. Gas samples were withdrawn from all elevated and one ambient CO_2 chamber at 4-min intervals at canopy height and adjustments to the elevated [CO_2] were made daily. Carbon dioxide concentration, determined by an absolute CO_2 analyzer (Li-Cor 6252, Li-Cor Corp., Lincoln, NE USA),

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Abbreviations: AHI, apparent harvest index; ai, active ingredient; DAS, days after sowing.

indicated average daytime [CO_2] (0600-1900 h) values of 401 ± 21 , 384 ± 14 , and average nighttime values of 542 ± 35 , 527 ± 26 $\mu\text{mol mol}^{-1}$ for the ambient CO_2 treatment in 2003 and 2004, respectively, and corresponding CO_2 values of 624 ± 18 , 631 ± 23 (daytime) and 745 ± 32 , 753 ± 38 $\mu\text{mol mol}^{-1}$ (night-time) for the elevated CO_2 treatment over the same time period.

Growth Conditions

Integrated day-time micrometeorological conditions of photosynthetic photon flux indicated that the chamber transmitted -90% of all incoming light, with an average daytime temperature increase of 0.8 and 1.3°C above the outside ambient temperature for 2003 and 2004, respectively. Overall, average temperatures during the growing season were 1.1°C below and 0.4°C above the 100-yr average for Maryland in 2003 and 2004, respectively. Precipitation values for this same period were 833 and 543 mm. The 2003 year was the wettest in Maryland since the onset of record keeping in 1895.

Before planting and chamber placement, the top 20 cm of soil was removed over the experimental field, bulked, and mixed for each year of the experiment. Subsamples placed in flats indicated uniform mixing, as determined by germination, and the subsequent presence of approximately 25 different annual and perennial weeds. Following chamber placement, soybean 'Ascro' (Ag3002, 'Round-up Ready', Maturity Group III, determinate) was planted within the chambers and in border rows surrounding the chambers on 27 June and 14 May for 2003 and 2004, respectively. The later planting date in 2003 was necessitated by excessive moisture during May and early June (i.e., the presence of standing water in the field plots during this period). Row widths were -30 cm with all plants thinned to 1 plant per 10 cm of row following emergence.

Timing of glyphosate application coincided with the period just before canopy closure of soybean rows (as per the recommendations of the Maryland Cooperative Extension Service). Glyphosate was applied as a isopropylamine salt with standard surfactant. Spraying occurred approximately 54 and 48 d after sowing (DAS) for 2003 and 2004, respectively, for half of the experimental chambers (i.e., three ambient and three elevated). A pressurized backpack sprayer was used to apply 2.24 kg ai ha⁻¹ to each of the treated plots. The other six

plots received water only. No effort was made to control weeds on the water sprayed plots.

Vegetative and Reproductive Measurements

Soybean was considered mature when >95% of the leaves had senesced and pods were noticeably brown. Because of differential planting dates (because of the high precipitation in 2003), maturity occurred by late October and late September for 2003 and 2004, respectively. At maturity, four center rows from each chamber (i.e., excluding border rows) were cut at the base of the plant and harvested. At harvest, individual pods were counted and separated by treatment. Pods were air-dried and aboveground shoot dry matter (i.e., stems, petioles, peduncles) was oven-dried at 65°C for 72 h or until a constant dry weight was observed, then weighed. Pods were threshed by hand and seed collected and weighed. A subsample of 50 seeds was used to determine individual seed mass and to estimate seeds per pod. Because of leaf senescence and drop, harvest index was calculated as the ratio of seed mass to the sum of stem plus pod mass at maturity. This is typically done for commercial soybean and is referred to as the apparent harvest index (AHI) (Schapaugh and Wilcox, 1980).

Weed species were identified by chamber just before application of either water or glyphosate and again at soybean harvest. At soybean harvest, weeds within the harvested rows were cut at their bases, sorted into three general categories: C₃ broadleaf, C₄ broadleaf, or C₄ grass (no C₃ grasses were observed), dried, and weighed. No new species were observed between glyphosate application (i.e., canopy closure) and harvest. C₃ broadleaves were composed almost entirely (>95%) of three species; lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.) and Virginia copperleaf (*Acalypha virginica* L.); C₄ broadleaves were exclusively redroot pigweed (*Amaranthus retroflexus* L.) and C₄ grasses consisted of barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv.], Bermuda grass [*Digitaria sanguinalis* (L.) Scop.] and foxtail (*Setaria* spp.).

Statistical Analysis

The experiment was arranged in a completely randomized block at the field site with three replications of [CO_2] with and

Table 1. Averages and level of statistical significance of the one-way analysis of variance for CO_2 concentration (380 or 630 ($\mu\text{mol mol}^{-1}$)) effects on vegetative and reproductive characteristics of field-grown Round-up Ready soybean with and without applications of glyphosate (+Gly or -Gly) in 2003 and 2004.

Variable		Averages				Level of significance		
	Units	380	630	+Gly	Gly	CO ₂ effect	Gly effect	Gly X CO ₂
2003								
Stem weight	gm ²	135.7	195.3	181.0	149.9	*	*	
Pod number	#nT ²	1055	1155	1402	808	**		
Pod weight	gm ²	544.6	518.0	678.8	383.8	**		
Seeds/pod		1.7	2.1	1.7	2.0			
50 seed weight	g	8.5	7.0	7.5	8.2	*		
Total seed	gm -	299.3	329.9	393.3	235.8	*		
AHI		0.47	0.44	0.46	0.40	*		
2004								
Stem weight	gm ²	338.4	500.7	631.4	207.7	*	***	
Pod number	#nT ²	823	1089	1593	320	*	***	{*}
Pod weight	gm ²	385.1	479.9	764.4	100.7	***		
Seeds/pod		2.2	1.8	23	1.8			
50 seed weight	g	6.8	7.7	6.5	8.0	(*)		
Total seed	gm ²	285.8	365.8	590.2	61.4	*	***	
AHI		0.32	0.28	0.37	0.18	*	**	

(*) Indicates significance at $P < 0.10$.

* Indicates significance at $P < 0.05$.

** Indicates significance at $P < 0.01$.

*** Indicates significance at $P < 0.001$.

.t AHI is apparent harvest index, the ratio of seed mass to the sum of stem plus pod mass at maturity.

Table 2. Averages and level of statistical significance of the one-way analysis of variance for CO₂ concentration (380 or 630 $\mu\text{mol mol}^{-1}$) effects on above-ground biomass for weed species associated with field-grown Round-up Ready soybean with and without applications of glyphosate (+Gly or -Gly) in 2003 and 2004. Data are g per m².

Weed type	Averages				Level of significance		
	380	630	+Gly	-Gly	CO ₂ effect	Gly effect	Gly \times CO ₂
2003							
C ₄ grasses	138.1	128.3	0.0	266.1		***	
2004							
C ₃ broadleaf	48.3	303.9	18.5	333.7	***	***	***
C ₄ broadleaf	166.1	257.1	0.0	423.2		***	
C ₄ grasses	157.1	23.3	0.5	179.8	(*)	*	

(*) Indicates significance at $P < 0.10$.

* Indicates significance at $P < 0.05$.

*** Indicates significance at $P < 0.001$.

without glyphosate application (three replications \times two [CO₂] \times two glyphosate treatments). Vegetative and reproductive characteristics were determined for each year of the experiment by a two-way ANOVA with [CO₂] and glyphosate as the classification variables (Statview, Cary, NC, USA). Treatment comparisons were made using a Fisher protected least significant difference. Unless otherwise mentioned, differences for any measured parameter were determined as being significant at the $P \leq 0.05$ level.

RESULTS

Increasing the [CO₂] by $\sim 250 \mu\text{mol mol}^{-1}$ resulted in consistent increases in above-ground vegetative biomass, particularly stem weight, for soybean for both seasons (Table 1). However, the effect of elevated [CO₂] on seed yield (with glyphosate application) was incon-

sistent. Although individual seed weight tended to increase with [CO₂], the effect of [CO₂] was only observed for seed yield in 2004, primarily as a result of increased pod number (Table 1). Overall, the impact of elevated [CO₂] was greater on stem weight than seed yield, with a subsequent reduction in AHI (significant in 2004) (Table 1).

With respect to weed biomass and weed species, 2003 resulted only in the appearance of C₄ grasses; no effect of [CO₂] was observed on their growth (Table 2). In contrast, in 2004 an increase in total weed biomass was observed relative to 2003; and, a greater variety of weed species were observed including C₄ grasses, C₃, and C₄ broadleaf weeds. In 2004, a significant effect of [CO₂] treatment was also observed for the presence of either C₃ broadleaf and C₄ grasses ($P = 0.07$) and subsequent weed biomass (Table 2, Fig. 1). However, the relationship between increasing weed biomass and soybean seed yield was not affected by CO₂ treatment for either year (i.e., no significant differences in slope were observed, Fig. 2).

Not surprisingly, glyphosate application reduced or eliminated weed biomass with a subsequent increase in soybean yield parameters (with the exception of individual seed weight and seeds per pod, Tables 1, 2). No consistent interactions between glyphosate application and CO₂ concentration were observed for any yield parameter. In 2003, application of glyphosate resulted in 100% control of C₄ weeds irrespective of [CO₂] treatment. In contrast, in 2004, glyphosate application only resulted in 100% control for the ambient [CO₂] treatment (Fig. 3). Appreciable amounts of weed biomass (C₃ broadleaves) were still recorded after glyphosate application at the elevated [CO₂] treatment, resulting

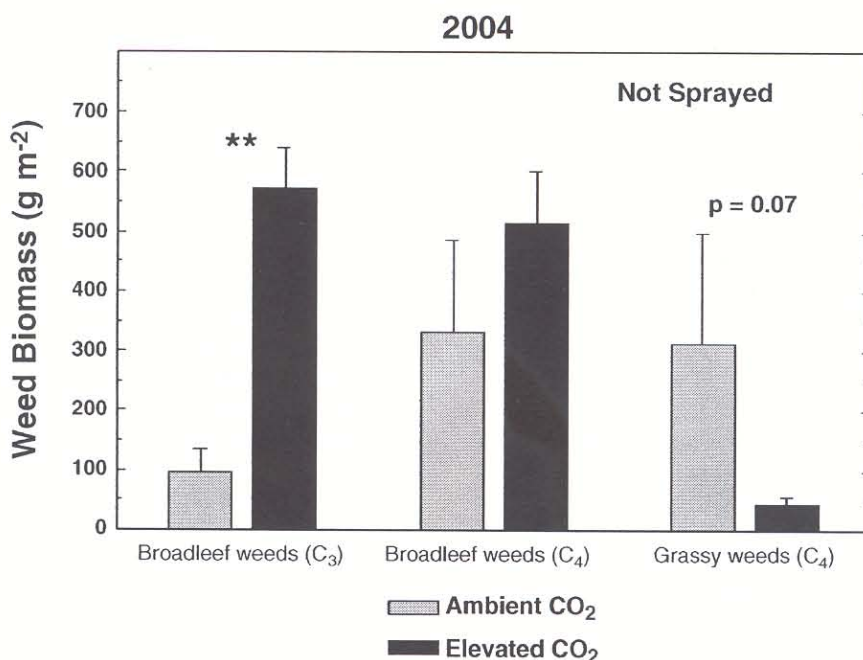


Fig. 1. Quantification of above ground weed biomass in three general categories, (C₃ broadleaf, C₄ broadleaf and C₄ grass) when grown at ambient and elevated ($+250 \mu\text{mol mol}^{-1}$) [CO₂] in Round-up Ready soybean without glyphosate application in 2004. Variation for a given weed category was tested by a one-way ANOVA, with three replicates. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

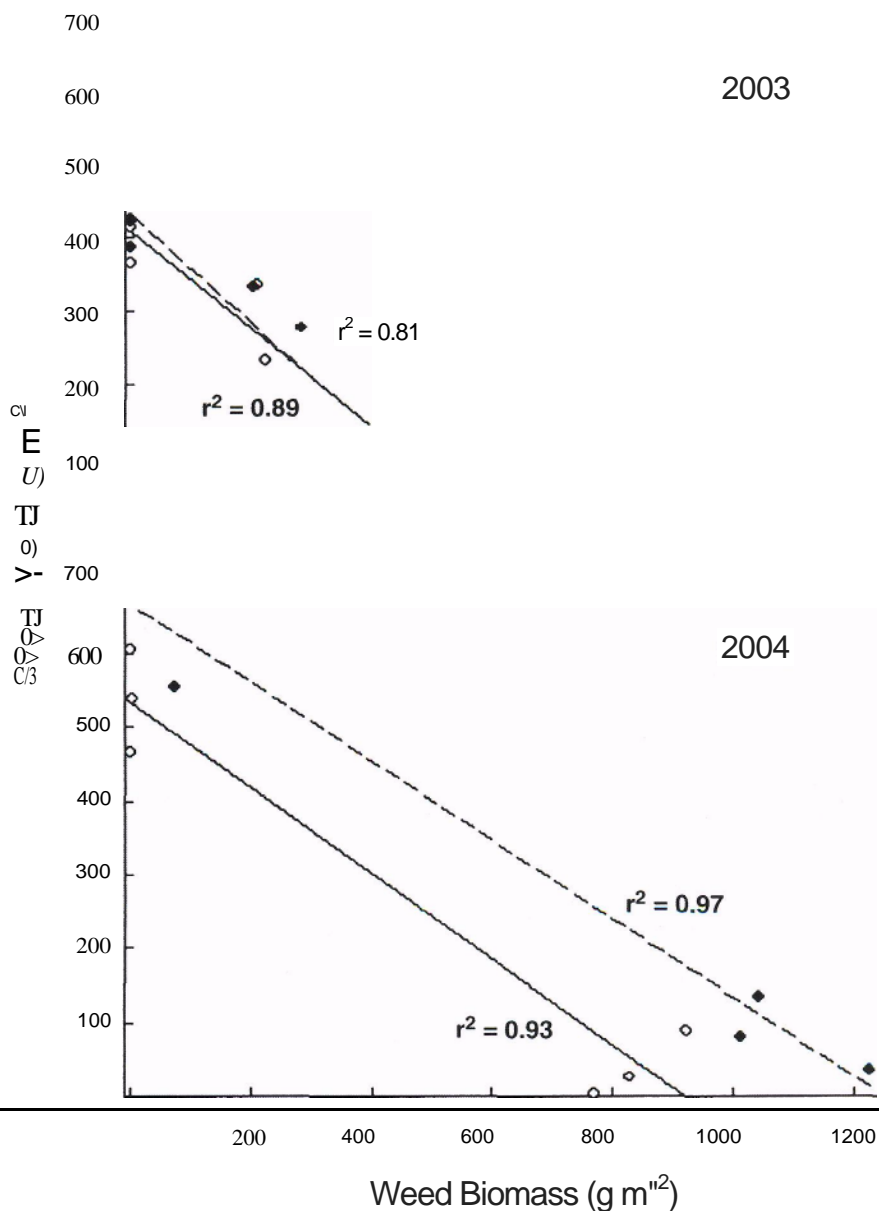


Fig. 2. Soybean seed yield (g m^{-2}) as a function of increasing weed biomass at either ambient (solid line) or elevated (ambient +250 $\mu\text{mol mol}^{-1}$, dashed line) CO_2 for 2003 and 2004. No differences in the slope of the regression as a function of $[\text{CO}_2]$ were observed (ANCOVA). in a significant $[\text{CO}_2]$ by herbicide interaction (Table 2, Fig. 3).

DISCUSSION

Although Round-up Ready soybean demonstrated a positive vegetative response to a $[\text{CO}_2]$ increase of -250 (xmol mol^{-1}) at maturity in both years, a significant effect on seed yield was only observed in 2004. For that year, the increase in seed yield was accompanied by a reduction in AHI, suggesting that vegetative growth may be a greater sink for additional carbon than reproductive growth. The decline in AHI for soybean observed here was consistent with previous work (Baker et al., 1989; Ziska et al., 2001) and supports the conclusion by Ainsworth et al. (2002) that soybean may show a reduction in AHI regardless of cultivar, growth habit, or maturity group.

If glyphosate (Round-up) is not applied, how does increasing $[\text{CO}_2]$ alter the growth of weed populations within the soybean canopy? Given that weed seeds were uniformly distributed within the seedbank before the start of each field season, the impact of CO_2 on weed populations may be dependent on those environmental factors that altered the establishment of C_3 and C_4 weeds. One such factor may be precipitation, which is generally recognized as a significant environmental factor in weed establishment, i.e., higher precipitation favors anoxic conditions and the establishment of shallow rooted grasses or adapted species (Patterson, 1995b). This is consistent with observations from the first year of the current study: specifically, that high precipitation

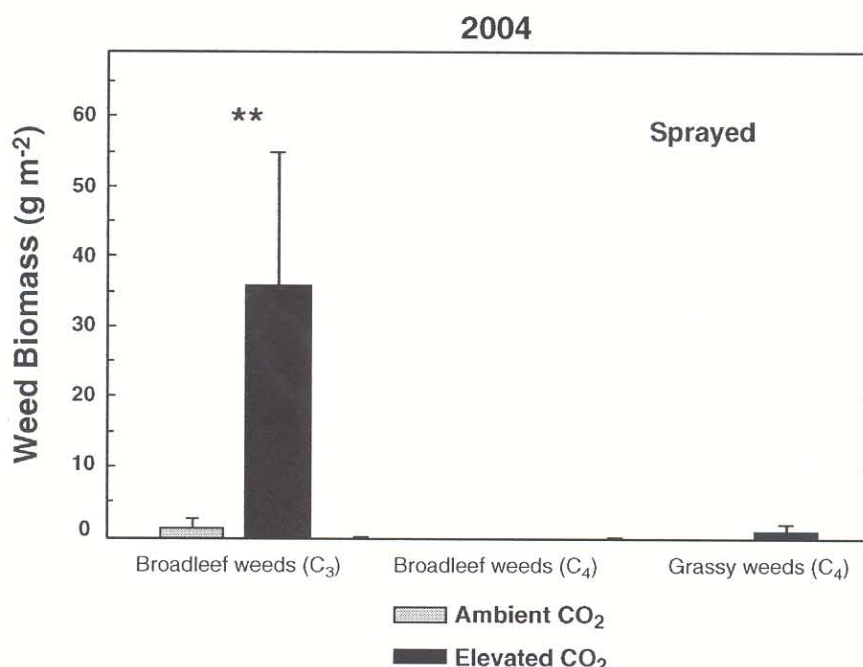


Fig. 3. Quantification of above ground weed biomass in three general categories, (C₃ broadleaf, C₄ broadleaf and C₄ grass) when grown at ambient and elevated (+250 $\mu\text{mol mol}^{-1}$) [CO₂] in Round-up Ready soybean, but after application of recommended amounts of glyphosate (Round-up). Note that significant amounts of C₃ broadleaf weeds were still present at the elevated CO₂ treatment after glyphosate application. Variation for a given weed category was tested by a one-way ANOVA, with three replicates. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

rates (the highest recorded in Maryland since 1895), resulted in the sole recruitment of C₄ grasses. In this circumstance, elevated [CO₂] had no effect on weed biomass at maturity. However, for near normal precipitation in the following year, a range of weed species, including C₄ broadleaf and grasses, and C₃ broadleaves, was observed. In this year, a significant effect of elevated [CO₂] on total weed biomass was noted, due primarily to an approximate 5× increase in the amount of C₃ broadleaf biomass relative to ambient conditions.

How do differences in [CO₂] alter weed-crop competition and crop losses? The decrease in seed yield per increase in weed biomass (i.e., the slopes in Fig. 2) did not vary between years or as a function of [CO₂], suggesting that reductions in soybean productivity were not significantly altered by weed species per se. This has been observed previously for field grown soybean in competition with a C₃ and C₄ weed population (Ziska, 2000).

But is weed-crop competition even likely if weeds are controlled chemically? Commercially, one of the advantages of using a genetically modified crop such as Round-up Ready soybean is the nonselective application of herbicides for weed control. Previous experimental data have indicated that the effectiveness of glyphosate could be reduced for individual C₃ weeds at elevated [CO₂] under glasshouse conditions (Ziska et al., 1999; Ziska and Teasdale, 2000); however, it was uncertain if similar results would be obtained at commercial application rates in situ.

In the current field study, the overall efficacy of glyphosate application in response to elevated [CO₂] was reduced in 2004. Could greater growth of soybean in response to elevated [CO₂] be reducing spray coverage of glyphosate? This seems unlikely since elevated [CO₂]

resulted in greater vegetative biomass in both 2003 and 2004. Alternatively, previous research on individual plants suggested that reduced glyphosate efficacy at elevated [CO₂] was associated primarily with C₃ weeds (Ziska et al., 1999). Such a finding is consistent with the reduction in efficacy observed concurrently with the stimulation of C₃ weeds in field grown soybean for 2004.

If reduced chemical efficacy in response to rising [CO₂] is a function of the relative proportion of C₃ vs. C₄ weeds, then the current study also suggests that those environmental factors that influence the ratio of C₃:C₄ species could play a role in [CO₂] response and subsequent chemical efficacy. For example, if high precipitation results in anoxic conditions and greater grass formation (with an over-representation of the C₄ pathway), the effect of [CO₂] on plant growth could be minimal and glyphosate efficiency unimpaired. Alternatively, if high soil nitrogen increases the population of C₃ relative to C₄ species, then the impact of [CO₂] may be considerable, with subsequent reductions in chemical efficacy.

The mechanistic basis for the reduction in glyphosate efficacy at elevated [CO₂] for C₃ species has not been entirely explained. Previous work with lambsquarters (C₃ broadleaf) suggested that CO₂ induced increases in biomass, while a factor, did not entirely account for the reduction in chemical efficacy (Ziska et al., 1999). Recent work with Canada thistle [C₃ broadleaf, *Cirsium arvense* (L.) Scop.] grown in monoculture under field conditions indicated that in addition to growth stimulation, a greater root to shoot ratio and subsequent below-ground dilution of glyphosate increased glyphosate tolerance at elevated relative to ambient [CO₂] (Ziska et al., 2004). In any case, differences in plant size, absorbance

regarding sampling of weeds within the soybean canopy and physical disturbance effects on soybean seed yield.

But even if increasing $[\text{CO}_2]$ alters glyphosate efficacy, is there cause for concern? It could be argued that chemical management could adapt to any CO_2 induced changes in weed control. For example, glyphosate could be applied earlier in the season, or alternatively, herbicide concentration or spraying frequency could be increased. However, if glyphosate application is too early (i.e., before canopy closure), then weed regrowth could occur; similarly, changes in the frequency of application or concentration of glyphosate, while increasing weed control, would also increase economic and/or environmental costs. From an economic perspective, it may be worth noting that any profits associated with greater seed yield at elevated $[\text{CO}_2]$ could, potentially, be offset by additional costs of weed control.

While the response of agronomic species to rising atmospheric $[\text{CO}_2]$ has been confirmed in literally hundreds of studies (e.g., Kimball, 1983), it is becoming increasingly evident that $[\text{CO}_2]$ will also benefit agronomic and invasive weeds as well (Ziska and George, 2004). The argument that rising atmospheric $[\text{CO}_2]$ will reduce weedy competition because the C_4 photosynthetic pathway is over represented among weed species (e.g., Holm et al., 1977) does not consider the range of available C_3 and C_4 weed species present within the agronomic seed bank, nor those environmental factors (e.g., precipitation) that may influence their relative proportion following emergence. Overall, the data presented here suggest that, depending on weed species (C_3 vs. C_4), elevated $[\text{CO}_2]$ can increase weed biomass, decrease yields, and reduce glyphosate efficacy for Round-up Ready soybean.

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